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APPENDIX 1

SOLUTIONS

1. Solution I (Alkaline lysis method, TEG)
   Tris (pH 8.0 ) 25mM
   EDTA 10mM
   Glucose 50mM
   Store at 4°C

   Solution II
   SDS 1%
   NaOH 0.2N
   Prepared fresh and stored at room temperature

   Solution III
   5M Potassium acetate (pH 4.8)
   To 60ml of 5M potassium acetate, added 11.5ml of glacial acetic acid and
   28.5ml of DD water. The resulting solution is 3M with respect to potassium and
   5M with respect to acetate.

2. Qiagen Plasmid preparation

   Buffer P1
   RNase A 100µg/ml
   EDTA pH 8.0 10mM
   Tris HCl 50mM
   Store at 4°C

   Buffer P2
   NaOH 200mM
   SDS 1%
   Store at room temperature

   Buffer P3
   Potassium acetate pH 4.8, 2.55mM
   Store at room temperature
Buffer QBT
NaCl 750mM
MOPS pH 7.0 50mM
Ethanol 15%
Triton-X 100 0.15%
Store at room temperature

Buffer QC
NaCl 1.0M
MOPS 50mM
Ethanol pH 7.0 15%
Store at Room Temperature

Buffer QF
NaCl 1.25M
MOPS 50mM
Ethanol pH 8.2 15%
Store at room temperature

3. 0.5M EDTA pH 8.0
46.2 g of Di Sodium EDTA.2H$_2$O was dissolved in 200 ml H$_2$O. Adjust pH with NaOH and make up the final volume to 250ml. Stirred vigorously to dissolve and autoclave.

4. 5M Potassium Acetate (pH 5.2)
To 60ml of 5M potassium acetate, added 11.5ml of Glacial Acetic acid and add DD water to a final volume of 100ml. The resulting solution is 3M with respect to potassium and 5M with respect to acetate.

5. 1M Tris-HCl
Dissolve 121.1 g of Tris-base in 800ml of DD water. Adjust the pH to the desired value by adding concentrated HCl. Allow the solution to cool to room temperature before making final adjustments of pH. Make up the final volume to 1000ml.
Autoclave and store at room temperature.

6. Denaturing solution
NaOH 0.5M
NaCl 1M

7. Neutralizing Solution
Tris HCl, pH 7.4 0.5M
NaCl 3.0M

8. 100X Denhardtts (For 500 ml)
Ficoll 10gm
Poly-vinylpyrrolidone 10gm
Bovine Serum Albumin 10gm
Dissolve in H2O and store at room temperature.

9. 20X SSC pH 7.5
Tri Sodium Citrate 88.2gm
NaCl 175.3gm
Dissolve in 900ml of DD water, adjust the pH to 7.5 and make up the final volume to 1000ml. Autoclave and store at room temperature.

10. 20 X TBE pH 8.0 (2000ml)
Tris HCl 431.12
Boric acid 220.12
EDTA 29.76

11. 10 X PCR buffer
TAPS 100mM
MgCl2 20mM
KCI 500mM
Gelatin 0.1%
12. **Sheared Salmon Sperm DNA**

SSS DNA (Sigma type III Sodium salt) was dissolved in water at a concentration of 10mg/ml. If necessary, stir on magnetic stirrer for 2 to 4 hours. Adjusted the solution to 0.1M NaCl and extracted once with phenol:chloroform. The aqueous phase was recovered and the DNA sheared by passing through a 17gauge needle. Precipitated the DNA by adding 2 volumes of ice-cold ethanol and recover by centrifugation. Redissolved at a concentration of 10mg/ml.

13. **Luria-Bertani Medium (LB Medium)**

To 950ml de-ionized H₂O added:

- Bacto tryptone: 10 g
- Bacto yeast Extract: 5 g
- NaCl: 10 g

Shook until the solutes had completely dissolved. Adjusted the pH to 7.0 with 5N NaOH. Made up the final volume to 1000ml. Autoclaved and stored.

To prepare LB-agar medium, added agar to a final concentration of 1.5%. Autoclaved and stored.

14. **Prehybridization Buffer for radioactive probe**

- 20XSSC: 25ml
- 100X Denhardts: 2ml
- 1M Tris pH 8.0: 5ml
- 10% SDS: 2ml
- 0.5M EDTA: 2ml
- Salmon sperm DNA (5mg/ml): 2ml
- H₂O: To make 100ml

15. **CTAB Extraction Buffer**

- NaCl: 0.7M
- Tris HCl pH 8.0: 50mM
- EDTA pH 8.0: 10mM
- CTAB: 1%
- β-Mercaptoethanol: 0.1%
16. **10% CTAB (For 200ml)**
Dissolved 20 g CTAB in 200 ml of 0.7 m NaCl solution. Store at room temperature.

17. **1% CTAB (For 2000 ml)**
- CTAB 20.0gm
- Tris HCl pH 8.0 12.12gm
- EDTA pH 8.0 7.44gm
Dissolved in 1500ml DDwater, adjusted the final volume and sterilized by autoclaving. Stored at room temperature.

18. **IPTG (Isopropyl-β-D-thiogalactopyranoside, C₉H₁₆O₅S; Molecular weight 238.3) 25mg/ml.**
Dissolved appropriate amount in DD water Filter sterilized and stored at -20°C.

19. **X-Gal (5-Bromo,4-Chloro, 3-indolyl- β-D-Galactopyranoside, C₁₅H₁₅BrClNO₆; MW 408.6)**
Dissolved appropriate amount in Di-methylformamide to a concentration of 20mg/ml. Stored in dark coloured bottles at -20°C.

20. **Ampicillin Trihydrate (10mg/ml)**
Dissolved the required amount in MQ water with the help of a few drops of NaOH. Filter sterilized and stored at -20°C.

21. **M-9 (Minimal Medium, 1000 ml)**
To 800ml of sterile deionized water that was cooled to 50°C added 200ml of 5X minimal salt. Before pouring the plates added necessary amino acids and carbon sources.

**5X Minimal Solution (1000 ml)**
- Na₂HPO₄.7H₂O 64 g
- KH₂PO₄ 15 g
- NaCl 2.5 g
- NH₄Cl 5.0 g
Aliquoted and stored after autoclaving.
Carbon source and amino acid. For 1000 ml of M9 medium added

- 20% Glucose 1ml
- 20% MgSO₄ 100μl
- B.Thiamine (1mg/ml) 500μl
- Amino Acid* (4mg/ml) 1ml

* For DH5α- used amino acid arginine
* For NM522- used amino acid proline

22. RNaseA Solution
Dissolved Pancreatic RNase (RNase A) at a concentration of 10mg/ml in 10mM Tris HCl pH 7.5, 15mM NaCl. Boil at 100°C for 15 min. Stored at -20°C.

23. 5% Polyacrylamide Gel

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>21.0 g/20ml</td>
</tr>
<tr>
<td>40% Acrylamide</td>
<td>6.0ml</td>
</tr>
<tr>
<td>20X TBE</td>
<td>2.5ml</td>
</tr>
<tr>
<td>H₂O</td>
<td>To make 50 ml</td>
</tr>
</tbody>
</table>

Filtered through Whatmann 1MM and stored in a dark bottle.

40% Acrylamide Solution
Dissolved 38 g of Acrylamide and 2 g of Bis-acrylamide in 100 ml of DD water. Stored in a dark bottle at 4°C.
Bind silane was prepared by mixing 75 μl of bind silane (γ-methacryloxy propyl trimethoxy silane) to 5ml of 10% acetic acid (v/v).

24. Repel Silane
Diluted Repel-silane stock (100%) to 3 to 4% in carbon tetra chloride for use. Stored in a dark bottle at 4°C.

25. 10% Ammonium persulphate
Dissolved 100mg of ammonium persulphate in 1ml of DD water. Store at 4°C.
26. **1M CaCl₂**
Dissolved 54 g of CaCl₂·6H₂O in 200ml of DD water. Sterilized by autoclaving and stored at 4°C.

27. **Ethidium Bromide (10mg/ml)**
Dissolved 1g of ethidium bromide in 100ml of DD water with the help of a magnetic stirrer till the dye had completely dissolved. Stored the solution in a dark bottle at 4°C.

28. **3M Sodium acetate (pH 5.2)**
Dissolved 408.1g of sodium acetate·3H₂O in 800ml of DD water. Adjust pH to 5.2 with glacial acetic acid. Dispensed in 100ml aliquots. Autoclaved and stored at room temperature.

29. **DNA extraction buffer (Dellaporta method)**
- Tris-HCl pH8.0 100mM
- EDTA pH8.0 50mM
- NaCl 500mM
- β-mercaptoethanol 0.1%
Sterilized by autoclaving and stored at room temperature. Added β-mercaptoethanol just before use.

30. **Homogenizing buffer (HB)**
- Sucrose 300mM
- MgCl2 5mM
- Tris pH7.8 50mM
Filter sterilized and stored at 4°C

31. **HBT**
- HB 98ml
- Triton X-100 2ml
Tris pH 8.0  50mM
EDTA pH 8.0  50mM
NaCl  50mM
Sarkosyl  2%

33.  **TE (Tris-EDTA)**
Tris-HCl pH 8.0  10mM
EDTA  1mM

34.  **10X gel loading buffer**
Xylene cyanol  10%
Bromophenol blue  10%
EDTA  150mM
Glycerol  70%

35.  **Nuclei buffer**
Sucrose  0.3M
Tris-HCl pH-8.0  10mM
EDTA  1mM

36.  **DNA extraction buffer (Nuclei method)**
Tris-HCl pH 8.0  100mM
NaCl  500mM
EDTA  20m M
SDS  1.0%
β-mercaptoethanol  0.1%
Add 4mg/ml of Sodium di ethyl di-thio carbamate just before use.

37.  **Proteinase K (10mg/m)**
Dissolved Proteinase K at a concentration of 10mg/ml in DDwater. Filter sterilize and store at -20°C.
38. **Buffer A1 (Jetsorb®)**
   - NaClO₄
   - TBE solubilizer
   - Sodium acetate

39. **Buffer A2 (Jetsorb®)**
   - 70% alcohol
   - NaCl
   - EDTA
   - Tris-HCl

40. **Esterification reagent**
   - Methanol 20ml
   - Benzene 4ml
   - Acetyl chloride 1ml